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## ON THE FEEDING REACTIONS AND DIGESTION IN THE CORAL POLYP *ASTRANGIA DANÆ*, WITH NOTES ON ITS SYMBIOSIS WITH ZOÖXANTHELLÆ.

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In comparison with the extensive literature on the feeding reactions and the digestion in actinians, these phenomena in madreporarian polyps have been studied by a few authors only. Our knowledge of these phenomena is chiefly due to Carlgren (1905, *Caryophyllia*), Duerden (1906, *Fungia* and *Favia*), Carpenter (1910, *Isophyllia*), and Vaughan (1912, various West Indian reef-corals). In the following pages the results are given of an investigation on the feeding reactions and the digestion in the coral-polyp *Astrangia danæ* Ag.

The work on which this paper is based was made possible by a subvention of the Netherland-America Foundation, which enabled me to visit the United States for some months. It was carried on in the Marine Biological Laboratory at Woods Hole (Mass.) in August and September, 1924. I want to thank here the Director of this Laboratory, Dr. F. R. Lillie, for the opportunity I obtained to work some time at this institution.

The study of the digestion in *Astrangia* is meant as a base for the further study on the digestion in the polyps of reef-corals. As is well known, the polyps of reef-corals contain a large number of unicellular algæ, the zoöxanthellæ, which are located in the entoderm-cells. Although this association of coral-polyps with algæ is generally considered as a kind of symbiosis, there are very few statements on the advantages which both organisms derive from it. One of the ways to investigate this problem is the comparison of the physiology of a form with symbiotic algæ with

that of a form without these organisms. Now *Astrangia danae* generally is completely devoid of zoöxanthellæ, but during the time I worked in Woods Hole I obtained some colonies which were strongly infected with these algæ. The fact that my material consisted partly of polyps without zoöxanthellæ and partly of those infected with the symbiotic algæ enabled me to make a comparison of the feeding and digestion in these different kinds of polyps. As I disposed during the first half of my time in the Marine Biological Laboratory only of polyps without zoöxanthellæ, the larger part of my experiments were made with such uninfected polyps. Afterwards, when comparing the phenomena of digestion in polyps without zoöxanthellæ with those found in uninfected polyps, I could at least partly investigate the rôle of the zoöxanthellæ in the feeding of the polyps which are infected by these algæ.

I kept the polyps of *Astrangia* easily alive in glass-vessels with running sea-water, and at the end of a month they were in the same healthy state as at the beginning of the experiments. Some colonies even had enlarged in size by budding. The fact that *Astrangia* may be kept alive for some time in artificial surroundings was already recognized by Agassiz (1850), who kept his specimens alive by changing the sea-water twice a day. This author also gives a description of the structure of the polyps and states that they can be fed with fragments of molluscs. Besides this he gives some notes on the digestion in *Astrangia*. According to Agassiz the food at first remains for some time in the upper part of the gastric cavity before it descends to the lower part of this cavity where it is mixed with water and distributed over the internal organs of the polyp. As may be seen in the following pages these notes on the digestion are not completely in accordance with my observations.

The description of the general form of the polyps by Agassiz is very accurate. He states that there are three cycles of tentacles, those of the first cycle are of a larger size than those of the second, whilst the tentacles of the third cycle are somewhat smaller than the others. At each extremity of the laterally compressed mouth a tentacle of the first cycle is found. Agassiz discerned two varieties in the species: one with white polyps and another in which the polyps were pink or rosy.

An account of the chief peculiarities of *Astrangia danæ*, accompanied by figures, some of which represent the polyps in an expanded form, are found in the publications of Mrs. and Mr. Agassiz (1865) and of Dana (1890). Verrill and Smith (1874) also give a short description of the polyps of this species. They state that the animals are white and that the polyps in expansion rise high above the skeleton. These authors also note that the polyps will feed readily upon fragments of molluscs or crustaceans.

In the literature on the coral *Astrangia* I have not found any remark on its symbiosis with zoöxanthellæ. In the greater part of the colonies of this species found in the Woods Hole region all polyps are completely free of zoöxanthellæ. This was invariably the case in the specimens which I obtained from the piles of the wharf at Woods Hole, in those dredged in the harbour between Woods Hole and Nonamesset Island and in those dredged to the southeast of Nonamesset Island from about 8 fathoms. On the other hand the specimens dredged to the southwest of Falmouth (south of the oyster-pond) were always more or less infected with zoöxanthellæ. Some of the polyps of this locality had a dark brown color owing to the multitude of yellow algæ occurring in their tissues, others had various lighter shades of brown or were almost white, but a microscopical examination of a part of their tentacles or oral disk proved that they invariably contained zoöxanthellæ. It is a strange fact that this symbiosis of the polyp with yellow cells in the Woods Hole region is restricted only to the colonies found in certain smaller localities.

The polyps which are not infected by zoöxanthellæ are quite colorless and these are therefore especially fit for the study of the feeding reactions as foreign bodies can be seen through the transparent tissues of the animals. Usually the skeleton has a greenish or light-red color, which is due to the occurrence of algæ living in the skeleton. Then at first sight the polyps sometimes seem to have a pink or greenish hue by the transparency of their tissues. Probably these red or green-colored algæ are only different stages in the development of one species as their microscopical structure is very much alike. As yet I have no positive evidence that they belong to one of the forms described by Duerden (1905). In some colonies a compact mass of algal matter is obtained after

decalcifying the specimens, in other colonies only sparsely distributed threads are to be found. In an allied species, *Astrangia solitaria*, the skeleton is also penetrated by boring algæ (Duerden, 1902). Besides these boring forms there is another alga which lives in the tissue of the polyps. Mrs. Dr. A. Weber van Bosse, who kindly examined the preserved material of these algæ, found that it represents a new species of the genus *Streblonema*.<sup>1</sup>

For different reasons the polyps of *Astrangia* are especially fit objects for the study of their feeding habits. During the daytime they are usually fairly well expanded.<sup>2</sup> After being disturbed the polyps contract within the skeleton, but usually they will expand again in a very short time after the disturbance. Even when they are transferred from one glass-vessel to another they soon return to their expanded state. One of the further advantages of *Astrangia* in my experiments was that the polyps will ingest almost every particle which is offered to them, as well food as non-nutrient objects.

All the polyps of freshly-collected colonies of *Astrangia danae* do not react in quite the same way on a certain stimulus, probably on account of the disturbance caused when they are collected. When, however, the colonies have been a few days in the laboratory under constant external influences they constitute a fairly uniform material for feeding experiments. Especially more or

<sup>1</sup> I am indebted to Mrs. Dr. A. Weber van Bosse for the following diagnosis of this interesting form:

"*Streblonema Willyae* n. sp.

Frondibus microscopicis in telum *Astrangiae danae* penetrantibus, compositis e filamentis sterilibus, irregulariter alterne aut secundatis ramosis, 2-5  $\mu$  latis, aggregatis, fasciculos prope superficiem hospitis formantibus. Chromatophoris taeniatis aut disciformibus, parietem cellulae non totius tegentibus. Sporangii ignotis. Gametangiis cylindricis aut fusiformibus, singulis aut ramosis in filamentis plerumque terminalibus, interdum lateralibus, longis 60-120  $\mu$ , latis 8-10  $\mu$ ; loculis uni- et pluriseriatis. Pili desunt.

This new species of *Streblonema* is so far interesting as it grows in a coral, not in the skeleton but in the soft tissue of the animal. It has this mode of living in an animal in common with the genus *Endodictyon*, but the apical and above all the branched gametangia seem to indicate that its proper place is in the genus *Streblonema*."

<sup>2</sup> It is a well-known fact that the greater part of the madreporarian coral polyps are nearly always found in a contracted state during the day-time. Besides *Astrangia* there are, however, exceptions on this rule, e.g., the polyps of *Fungia*, *Goniopora* and *Euphyllia* from the East Indian coral reefs, which are during daytime in a fully expanded state.



less hungry polyps are very susceptible to a certain stimulus, as is the rule in anthozoans. On the contrary well-fed polyps react very slowly on the same stimulus. Also in this respect *Astrangia* agrees with other anthozoans (cf. Jennings, 1905).

The chief results of my experiments on *Astrangia* are published in a preliminary paper (Boschma, 1925), in which especially the rôle of the zoöxanthellæ in the feeding of the polyps is compared with that in other anthozoans.

#### FEEDING REACTIONS.

The reactions of *Astrangia* to food and other substances are in many respects quite similar to those found in other representatives of the anthozoans. Before the results of my own investigations on *Astrangia* a short summary of the literature on this subject is given below.

Pollock (1883) was the first to describe that sea-anemones may perceive the presence of food-particles in their neighborhood, as this causes the opening of the mouth and movements of the tentacles of the animals. Loeb and Nagel some years afterwards worked out the reactions to food and other substances in actinians more in detail. According to Nagel (1892, 1894*a*, 1894*b*) the actinians have a sense of taste, which is located only in the tentacles, and the food is brought to the stomodæum by muscle-action. Nagel (1894*b*) already stated that hungry actinians also ingest morsels of paper besides food-particles, whereas well-fed ones will only take food. The results of Loeb (1891, 1895) differed in some respects from those of Nagel. The phenomenon which was called by Nagel the "sense of taste" is according to Loeb a reaction to chemical stimuli. Loeb showed that the tentacles are not the only organs in which the response to chemical stimuli is located, he stated that also ciliary actions plays a part in the feeding of actinians. Moreover by cutting an actinian into two halves Loeb proved that a part of such a polyp devoid of its tentacles even takes food more readily than a normal individual.

Parker (1896) confirmed Loeb's results that ciliary action is one of the chief factors in the feeding of actinians. Parker found that the cilia of the stomodæum generally beat outwards, but when food substances come into contact with these cilia they reverse

their movement. In subsequent papers (Parker, 1905*a*, 1905*b*) the same author has worked out this phenomenon in further details. He found that certain chemical substances can induce the cilia to reverse. A number of statements on the part of the tentacles and of the ciliary action in different actinians during the feeding reactions is described by Carlgren (1905). This author found that in some species of actinians the ingestion of food takes place chiefly by ciliary action (*e.g.* in the Protanthææ); in other forms, however, the tentacles are the chief organs for the capture and transportation of the food to the mouth (*e.g.* *Tealia*).

The fact that not all the individuals of a certain species of actinian react in the same way on the same stimulus, was demonstrated by Jennings (1905). The state of metabolism of the actinian is one of the most important factors determining the reaction to substances which come into contact with the tentacles or the oral disk. Thus hungry polyps often will ingest inedible matter, while well-fed ones will refuse the same material. Piéron (1906) also states that different individuals of one species behave differently towards the same stimulus.

Different species of actinians also behave in quite a different way. Some species of actinians in confinement would ingest only a very limited variety of food, *e.g.* *Eloactis*, which refused everything but living *Balanoglossus* and *Hydroides* (Hargitt, 1907). On the other hand many other species ingest besides food also indigestible material. Instances of this are recorded by Torrey (1905) for *Sagartia*, Fleure and Walton (1907) for *Tealia*, Parker (1896) and Allabach (1905) for *Metridium*. In the case of *Metridium*, Parker (1905), however, has expressed the opinion that the foreign matter (filter paper) was touched by the hands of the experimenter and therefore acted as a chemical stimulus. All of the above-named forms, as also *Actinia* (*cf.* van der Ghinst, 1906) are able to discern food-particles from inedible matter. The latter is usually refused and food particles are ingested.

Summarizing the data available in the literature and those of his own investigations Parker (1917) states that the different factors which are combined to bring about the feeding reactions in actinians are the following: secretion of mucus, ciliary action, the action of the neuromuscular apparatus of the tentacles, of the

oesophageal cilia, and of the transverse mesenteric muscles. Only the muscular reactions are under the control of the animal as a whole.

The feeding reactions of *Alcyonium* are described by Pratt (1906). In this form the tentacles are the chief organs for the capture of the food. The species exhibits a definite choice in selection of the food, as eggs of fish and of *Galathea* are not digested, whilst the polyps readily feed upon planktonic organisms and flesh of different fishes.

There are only a few papers dealing with the feeding reactions in madreporarian polyps. The first species, in which observations on the capture of the prey are recorded, is *Astrangia danaë*. Verrill and Smith (1874) mention that it catches its food with the tentacles, which afterwards transfer the food to the mouth. De Lacaze-Duthiers (1877) made some feeding experiments with *Caryophyllia Smithii* and *Balanophyllia regia*. In the former species the food, a piece of a living mollusk, placed on the oral disk, caused a depression of the disk in this place. By the action of the muscles of the oral disk it was now brought to the mouth. The tentacles remained quite inactive. In *Balanophyllia* on the contrary the tentacles pushed the food towards the mouth. After some time in both species the food was discharged through the mouth covered with mucus. These statements, however, can hardly represent the normal behavior of the polyps. Probably the animals were in an abnormal state, for De Lacaze-Duthiers had kept them during several years in captivity. Moreover these statements are not in accordance with those by Carlgren (1905) on *Caryophyllia*. According to Carlgren the tentacles of this species catch food-particles and deposit them on the central part of the oral disk. By ciliary movement they now are transported over the oral disk to the stomodæum and swallowed.

The polyps of *Siderastrea radians* seize the food, according to Duerden (1904), with the tentacles. According to the same author (Duerden, 1905) mucus plays an important part in the feeding reactions of *Fungia* and *Favia*. On the surface of the polyps a mucous layer is secreted. Small organisms which come into contact with the oral surface of the polyps are imbedded in the mucus, which is afterwards ingested.

Carpenter (1910) described the feeding reactions of *Isophyllia*. The tentacles of this species catch small planktonic organisms. When a certain amount of food is taken by the tentacles the sphincter of the edge-zone of the oral disk contracts and the oral disk then forms a kind of roof over the mouth and the surrounding parts. In the superficial chamber formed in this way the digestion of the food takes place.

Vaughan (1912, 1919) studied the reactions to food in *Maandra areolata* and many other West Indian reef-corals. The food is ingested through the combined action of ciliary movements, secretion of mucus, and the movements of the tentacles. In some species, e.g., in *Orbicella cavernosa*, the mesenterial filaments are often protruded through the column wall and can catch food and even digest it whilst remaining outside of the gastric cavity.

With my feeding experiments on *Astrangia* I could confirm the statements of former authors that it will readily take food in captivity. When not overfed the polyps even will ingest almost everything which is offered to them. The food-objects which I used in my experiments were the following: crab meat (the muscles of the legs of the spider crab, *Libinia caniculata* Say.), fish meat, the soft parts of mussels, different species of worms (e.g., *Naraganseta corallii* Leidy which lives burrowing in the skeleton of *Astrangia*, *Hydroides* and other polychæt worms), pycnogonids, small amphipods, larvæ of higher crustaceans, copepods, ctenophores, etc. Also juice of crab or mussel meat is readily ingested. Besides these food-particles indigestible objects are ingested by hungry polyps with the same avidity: coarse sand, powdered writing chalk soaked in sea-water, clumps of litmus, carmine and iron carbonate.

When crab meat or fish meat is mixed with some coloring matter (India ink, litmus, ammoniac carmine, neutral red) it is as readily taken as pure meat. This method enabled me to trace the way of the food in the internal organs after it had been ingested, the coloring matter was only used as an indicator.

These experiments with pieces of meat could not give results which represent the normal feeding reactions. In the gastric cavity of freshly collected specimens often remains of small organisms are found, especially appendages of small crustaceans.

To study the normal feeding reactions I therefore fed the polyps with planktonic organisms, chiefly copepods and larvæ of decapod crustaceans. It proved to be an advantage when the food was colored, then it could be seen through the transparent tissues when it was captured and swallowed. For this reason I used the method employed by Fischel (1908) for daphnids and copepods of fresh water. To the sea-water which contained the animals a few drops of a solution of neutral red were added by means of which the water obtained a light yellow color. The copepods and other organisms now absorbed the coloring matter and gradually acquired a bright red hue. Especially the different parts of the intestinal tract absorbed a great quantity of neutral red, but also immediately beneath the skeleton and in the appendages the coloring matter was stored. These colored copepods and larvæ of decapods are as readily taken by the polyps as colorless ones.

When some of these colored planktonic organisms are put into a small glass containing a colony of *Astrangia* with expanded polyps the capture of these animals may be easily observed. Every now and then one of the animals comes into contact with a tentacle of a polyp. Smaller copepods then as a rule are immediately captured, they seem to stick to the tentacle, undoubtedly by their being paralyzed by nematocysts. The tentacle then suddenly contracts more or less and bends over the oral disk in the direction of the mouth. The prey, however, is not brought directly to the mouth. The latter, with the central part of the oral disk, slowly increases in height as a conical protusion and this expanded part gradually moves in the direction of the prey. At last the tentacle with the copepod comes into contact with the mouth, the tentacle releases the prey and bends back to its original place. The captured copepod slowly slides down through the stomodæum into the gastric cavity, undoubtedly by ciliary action, and the mouth returns to the central part of the disk.

Larger copepods and larvæ of decapods, however, are not so easily captured. When they touch a tentacle they often swim away with a sudden jerk, the attack of the nematocysts seeming insufficient to paralyze them. Only when these animals bump heavily against a tentacle they are immediately caught. Their



struggling movements cause a number of neighboring tentacles to move towards them, so that they finally become completely enveloped in a number of tentacles. Then they are slowly transferred to the mouth in the above described way, but they often remain struggling heavily, even when going down the stomodæum. Usually, however, the tentacles keep these larger organisms in the same position for some time, and only after they have ceased to struggle they are transferred to the mouth.

These experiments with copepods and other small crustaceans show that the tentacles instantly react when they are touched by a free swimming animal. The oral disk on the contrary is quite indifferent to the contact of these planktonic organisms. Sometimes a small floating copepod may be seen sinking down and falling on the oral disk of a coral polyp. It can remain there for some time without calling forth any reaction of the polyp. Afterwards it can swim away unharmed unless it happens to touch one of the tentacles, in which case it immediately brings forth the capturing reactions of the tentacle.

In the case of the feeding experiments with crab meat or other non-moving material of food the movements of the tentacles are very slow. In expanded polyps the small morsels of meat strongly adhere to the tentacles to which it is offered, and usually some neighboring tentacles also bend towards the meat, more or less enveloping it. The mouth with the top of the conically expanded central part of the oral disk moves slowly towards the prey and the tentacles push the meat downwards into the stomodæum. Some tentacles often protrude into the stomodæum, pushing against the food-material. The mouth then closes and the food slowly passes down into the gastric cavity.

Generally after the feeding the polyps partly expand by raising their column often considerably above the skeleton. The tentacles also remain in a more or less expanded condition. This expansion of the polyps generally takes place as well after the feeding of meat as after the feeding of free-swimming organisms. In this state the polyps are almost indifferent to mechanical stimuli. Whilst a hungry polyp quickly contracts when gently touched by a forceps the polyps which have just fed do not react on the same stimulus. A further peculiarity is that often some



time after feeding air-bubbles are to be seen in the upper parts of the polyps, probably escaping from the prey which is being dissolved in the gastric cavity.

The reactions of hungry polyps of *Astrangia* to comparatively heavy objects, as diminutive pebbles and clumps of litmus, writing chalk or iron carbonate, are almost the same. These objects, when laid against a tentacle or on the oral disk, bring about the feeding reaction, consisting of the above described movements of the tentacles and the central part of the oral disk. The objects are almost invariably ingested and come closely in contact with the mesenterial filaments. The mouth closes after the ingestion and the polyp remains for some time, in my experiments varying between 20 and 95 minutes (usually lasting about 50 minutes), quite motionless. The first movement made after this time usually is the enlargement of the central part of the oral disk to a conical protusion. Then the mouth opens and the foreign object is seen moving slowly upwards in the gastric cavity. The underside of the object is in touch with the mesenterial filaments of the polyp, which seem to push against it. In the stomodæum in all probability the outward movement of the foreign object is caused by ciliary action, but often some mesenterial filaments are seen protruding in the stomadæum till the object has been removed from the inside of the polyp. In this way the non-nutrient particles are soon out of the gastric cavity, chiefly by the action of the mesenterial filaments. The object falls from the mouth on the oral disk and slides down on one side. Here the tentacles bend downwards and then the object is completely removed from the polyp.

The foreign objects which have been in the gastric cavity for some time are covered with a thin mucous layer. Undoubtedly the mucus has here a protective function, to avoid that noxious particles come into contact with the tissues of the polyp. This function of the mucus is already described by Gee (1913) in *Cribrina*. In *Astrangia* especially on the clumps of chalk which are removed from the gastric cavity the mucus is clearly visible. Often other particles imbedded in this layer are removed with the non-nutrient object. In this way very small quantities of remains of food may be obtained from the gastric cavity without

causing any injury to the polyp. I used this method to make out whether or not zoöxanthellæ were present in the gastric cavity of polyps the tissues of which contained these unicellular algæ.

Small pieces of filter paper rarely induce the polyps of *Astrangia* to feeding reactions. To avoid the absorption of organic substances (cf. Parker, 1905) the pieces of paper were not touched with the hands, but they were handled by clean instruments and put on the tentacles of a hungry polyp. For some time the small objects remain attached to the tentacles, to which they adhere more or less, but as a rule they soon fall down without causing the feeding reaction. In some cases, however, a few pieces of filter paper are ingested and afterwards, after about 50 minutes, removed from the gastric cavity in the above described manner. The difference in behavior of hungry polyps to comparatively heavy objects as small pebbles and pieces of filter paper in all probability is caused by the difference in weight. The heavy objects give a definite mechanical stimulus which immediately brings forth the feeding reaction, whilst the pieces of filter paper act as indifferent objects.

When carmine-powder soaked in seawater is distributed over expanded hungry polyps large quantities are ingested. There is no evidence of ciliary action on the oral disk, probably cilia are not to be found here. Soon after the carmine has dropped on the oral disk it is imbedded in mucus and large strains of this mixture of mucus and carmine can be seen gliding down the stomodæum through the inward beating of the cilia of the latter. Often a quantity of carmine is ingested by the cells of the mesenterial filaments, but it also occurs, especially when large quantities of carmine are present in the gastric cavity, that clumps of carmine mixed with mucus are removed from this cavity as non-nutrient particles.

The feeding reactions of *Astrangia* in general therefore consist chiefly of four actions of the polyps: muscular action of the tentacles, muscular action of the central part of the oral disk, secretion of mucus by the oral disk, and ciliary action of the stomodæum.

## DIGESTION.

In a great number of anthozoans, chiefly in actinians, the phenomena of digestion are at least partially investigated. The different authors who have worked on this subject do not agree in every detail, though the chief features of the digestion are fairly well known.

The principles of our knowledge of the phenomena of digestion in actinians and other coelenterates are chiefly due to Metschnikoff and Krukenberg. Metschnikoff (1880, 1882) found that small food-particles are ingested by the entoderm cells of the mesenterial filaments in an amoeboid way. They are imbedded in these cells and digested here (intracellular digestion). Claus (1881) maintained that the ingestion of foreign corpuscles in the mesenterial filaments of coelenterates was already described by him before Metschnikoff made mention of this fact. But in Metschnikoff's papers the phenomenon of ingestion was described in quite a convincing form, whilst Claus' previous investigations (Claus, 1874) only contained some notes on the question.

Krukenberg (1880) proved that the mesenterial filaments of actinians are organs of digestion and maintained that digestion takes place solely against these organs. According to this author the mesenterial filaments have to come in contact with the food, then a secretion of digestive fluid takes place and the food-particles are more or less dissolved (extracellular digestion)<sup>3</sup>. Krukenberg (1881, 1882*a*, 1882-86) was not inclined to attribute such an important function to the intracellular digestion of small particles as described by Metschnikoff. He was convinced that the filaments secrete a digestive fluid, though he was not able to demonstrate this fluid in a free state in the gastric cavity. He even expressed the opinion that the enzymes of the captured animals could be used by the actinians for their digestion (Krukenberg 1882*b*, 1882*c*).

The investigations of Willem (1892, 1893) made it probably that besides intracellular digestion also extracellular digestion of

<sup>3</sup> In the work of O. and R. Hertwig (1879/80) already the opinion is put forward that the mesenterial filaments secrete a digestive fluid. This was based only on the study of the histological structure of these organs. The definite proof of the digestive function of the mesenterial filaments is given by the physiological studies of Metschnikoff and Krukenberg.

the food takes place in actinians. The larger food-particles are more or less dissolved into smaller corpuscles by the secretion of a digestive fluid, and afterwards the small objects are ingested by the entoderm-cells as described by Metschnikoff. The ingestion of these small particles usually takes place in a zone of the mesenterial filaments in the neighborhood of the free edge, but after abundant feeding all the entoderm cells of the gastric cavity can ingest food, even those of the acontia. In the sea-anemone *Tealia* the soft parts of amphipods of the genus *Talitrus* are completely dissolved till only the bare skeleton is left (Willem, 1892). In siphonophores Willem (1894) found approximately the same phenomena: here also extracellular and intracellular digestion are present.

According to Chapeaux (1893) in the gastric cavity of actinians a free digestive fluid can be demonstrated. The secretion of this fluid occurs when food comes into contact with the mesenterial filaments. When the food is ingested in the entoderm cells the reactions of the food-vacuoles is acid. In siphonophores Chapeaux found that 15-20 hours after ingestion of food colored with litmus the vacuoles still had a red color. Chapeaux concluded from this fact that the digestive enzymes of actinians and siphonophores acted in an acid medium. The same opinion also is upheld by Metschnikoff (1893), Mesnil (1909) and Roaf (1910). On the other hand Jordan (1907*a*) supposed that after the acid reaction in the vacuoles an alkaline one would follow; the final resorption of the food probably would take place in the latter stage. The enzymes of the anthozoans then would present more likeness to those of other groups of invertebrates, and act in about the same way as the trypsin of vertebrates (cf. also Jordan, 1907*b*).

A number of investigations on digestion in actinians have been made to show whether there is only intracellular digestion in these animals or if there also is secreted a free digestive fluid which dissolves the larger corpuscles into small particles which can be ingested by the entoderm cells. According to Mesnil (1901) no free enzyme is secreted in the gastric cavity: the digestion in actinians is exclusively intracellular. Jordan (1907*a*), however, showed that food which could not come into contact with the

mesenterial filaments could be digested, and concluded that an enzyme causing extracellular digestion can be secreted by the mesenterial filaments. This opinion is also found in an article by Willem (1916), who maintains that extracellular digestion of the larger particles precedes the ingestion (phagocytosis) of the small particles, which are further dissolved by intracellular digestion.

The opinion of Biedermann (1911) on this question is in some respects an intermediate one between the two views dealt with above: he supposes that besides the intracellular digestion also the secretion of a digestive enzyme occurs. The reason why the enzyme is not found free in the gastric cavity (Mesnil, 1901) is according to Biedermann probably the following: only small quantities of digestive fluid are secreted by the mesenterial filaments, and only in immediate contact with the food.

Jordan (1913) upholds his original opinion, based on experiments with food packed in filter paper (Jordan, 1907a), which was digested in the gastric cavity of the polyps. This proves that the enzyme can easily penetrate through the whole of the food corpuscles and diffuse through every part of the gastric cavity where food particles are present. There is no special need for a contact of the food with the mesenterial filaments.

The principal organs of digestion in madreporarians are the mesenterial filaments, the same as in actinians. In *Cænopsammia* (*Dendrophyllia*) Gardiner (1900) found a small crustacean in one of the polyps, supported by the mesenterial filaments, which indicates that it was being digested here. In the mesenterial filaments of *Flabellum* the same author (Gardiner, 1904a) found fat globules and diatoms and other algal matter in the cells of the portion next to the edge which is crowded with nematocysts. When remains of partially digested food were found in the gastric cavity of madreporian polyps, these were lying against the mesenterial filaments, as recorded by Carpenter (1910) for *Isophyllia* (*Mussa*) and by myself (1924) for *Favia*. Moreover strong evidence for the digestive function of the mesenterial filaments is given by Vaughan (1912), who states that these organs in *Orbicella* may protrude through the column wall and catch and digest the food outside the gastric cavity.



In other groups of Anthozoa there are also a number of statements on the digestive function of the mesenterial filaments. In *Pennatula* and *Virgularia* Marshall and Marshall (1882) found foreign bodies embedded in the cells of these organs. Wilson (1883) has observed the ingestion of food by the mesenterial filaments of *Leptogorgia*, and the same author found diatoms and other solid foreign corpuscles enclosed in the mesenterial filaments of alcyonid polyps (Wilson, 1884). The most elaborate researches on digestion in octactinians have been made on *Alcyonium*. According to Hickson (1901) the mesenterial filaments of this animal secrete a digestive fluid which dissolves the food. The latter is afterwards ingested by the entoderm of the gastric cavity. These conclusions were confirmed by Pratt (1906). After elaborate feeding experiments on *Alcyonium* and the study of the changes in histological structure before and after feeding in the polyps of this form Pratt came to the same conclusion: in alcyonid polyps extracellular as well as intracellular digestion occurs. Before feeding the gland cells in the stomodæum and in the mesenterial filaments are filled with a secretion, after feeding they are empty. Pratt concludes that this secretion is mixed with the food in the gastric cavity and causes the partial dissolution of the larger objects. Afterwards the small particles are ingested by the cells of the mesenterial filaments.

The opinion of Dantan (1921) that the digestion of antipatharian polyps is only extracellular is solely based on the histological study of the one polyp in which food-particles could be found. This observation does not give sufficient evidence for the opinion that the polyps of this group are in respect to their digestion quite different from all other anthozoans.

In the mesenterial filaments of zoanthids also foreign bodies are found embedded in the cells (McMurrich, 1889, 1899). The zoanthid polyps therefore in all probability digest their food in the same way as other anthozoans.

As stated above, a great number of investigators found that the mesenterial filaments are the chief organs of digestion in anthozoans. In many of these statements the accurate place of the ingestion is said to be the part immediately behind the marginal zone which contains a great number of gland cells and nemato-

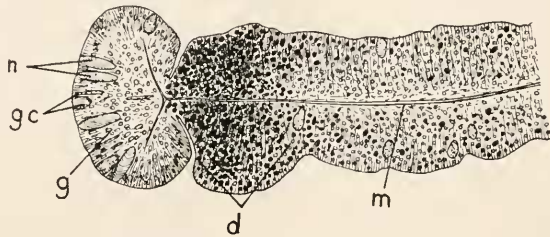


cysts. The mesenterial filament then consists of a marginal, glandular part and a part with digestive function next to the marginal part. Morphologically, however, the word "mesenterial filament" usually is employed in a more restricted sense, meaning only the marginal, glandular part (cf. Schneider, 1902). Also Duerden (1902), who states that the swollen part of the mesentery next to the marginal region is generally considered to be the principal seat of digestive function, denotes the marginal region only as the mesenterial filament. I use here the term in the same sense as other authors who studied digestion in anthozoans, and differently from the definitions of Schneider and Duerden. The glandular part of the mesenterial filament contains the bifurcation of the mesogloea. It is sharply separated from the digestive part by a deep furrow; the digestive part is, as in other madreporarians (cf. Duerden, 1902), not sharply separated from the rest of the mesentery, usually its thickness is slightly more.

In my studies on the digestion of *Astrangia* I chiefly used pounded crab meat thoroughly mixed with some coloring matter which served as an indicator after the food had been swallowed. A number of small colonies served as material for each experiment. I noted the time of feeding and studied the changes in the internal organs at various intervals by cutting open a polyp and examining a mesenterial filament under the microscope.

In the case of crab meat colored with India ink the food is readily taken by the tentacles and transferred to the mouth. Then it slowly descends through the stomodæum into the gastric cavity. By the transparency of the tissues it remains visible in the lower part of this cavity, where it is in contact with the mesenterial filaments. The polyps gradually expand more or less and often rise considerably above the skeleton (to about 15 mm., the tentacles also may stretch to the same length). When, after a very short time, the food is ingested into the mesenterial filaments these organs are visible as dark stripes through the wall of the polyps. Already one hour after feeding the digestive part of the mesenterial filament is crowded with small black spots (the particles of India ink) which indicate that the food with the coloring matter has been ingested here. Sparsely distributed black particles are also often found in the adjoining portion of the

mesentery. When food in great abundance has been offered to the polyps these parts ingest a great deal of it; when only moderate portions of food are given nearly all the food is ingested in the zone next to the marginal part. During a few days the distribution of the black particles in the mesenterial filaments does not alter perceptibly (Fig. 1); afterwards (about 5 days after



EXPLANATION OF THE TEXT FIGURE.

Transverse section of the free extremity of a mesentery of *Astrangia danae*, circa 2 days after the feeding of the polyp with a mixture of crab meat and India ink. Fixation 5 per cent. trichloracetic acid; stained with hematoxylin Delafield, *d*, digestive region of the mesenterial filament, crowded with black particles; *g*, glandular part of the mesenterial filament; *gc*, gland cells; *m*, mesogloea; *n*, nematocysts.  $\times 360$ .

feeding) the black particles gradually disappear from the digestive part of the mesenterial filaments. On the seventh day after the feeding usually the black particles have completely vanished from the digestive organs.

Crab meat mixed with ammonia carmine gave approximately the same results: during a number of days after the feeding the digestive zone of the mesenterial filaments then contains a quantity of red vacuoles.

Another series of feeding experiments was made with crab meat mixed with litmus. This mixture keeps its blue color whilst remaining in the gastric cavity, but after the food has been ingested in the mesenterial filaments the latter acquire a red color. This change of color is clearly visible in the living animal through its transparent tissues. Microscopic examination of a mesenterial filament soon after feeding proves that in the digestive zone a large number of red vacuoles of different size are present, whilst particles of litmus lying against the mesenterial filaments but not yet ingested have retained their blue color. Consequently the

digestive vacuoles possess an acid reaction whereas in the gastric cavity this reaction is neutral (or weakly alkaline).

During two days the digestive vacuoles keep approximately the same color. After about 48 hours a few vacuoles have acquired a bluish hue. Gradually the number of the blue vacuoles increases and after about three days the majority of the vacuoles in the digestive zone of the mesenterial filaments are blue. At last there are only a few red spots left, whilst the great number of blue vacuoles remain till about seven days after the feeding. During the greater part of the time the color is evenly distributed in the comparatively large vacuoles, but towards the end of the alkaline period (on the sixth and seventh day after the feeding) the coloring matter is concentrated to small particles, which gradually disappear from the mesenterial filaments.

In the digestion of *Astrangia* we can therefore distinguish two periods: an acid one, lasting for about two days, and an alkaline period during the rest of the time. In the later part of this alkaline period the excretion of the undigestible particles occurs. The changes in the reaction of the digestive vacuoles make it highly probable that the absorption of the food takes place in the alkaline period only. The acid period then is an antiseptic one (cf. Jordan, 1907b), in which noxious microorganisms are killed. The authors who stated that digestion of actinians takes place in an acid medium (cf. Chapeaux, Mesnil) did not extend their experiments for a sufficient time, otherwise they probably would have found that also in actinians the acid reaction is followed by an alkaline one. As compared with protozoans these two periods last a very long time. In *Paramecium* and *Colpidium* after feeding there is an acid period of  $5\frac{1}{2}$  to 70 minutes which is followed by an alkaline period of 1 to 30 minutes (Nirenstein, 1905).

One of the arguments for the presence of extracellular digestion in actinians results from the experiments of Willem (1892) referred to above. In *Astrangia* I have employed approximately the same method to demonstrate the evidence for extracellular digestion. I therefore studied the digestion of large copepods by the polyps. The copepods were vitally stained with neutral red after Fischel's method. At various intervals after the feeding I

opened a polyp and noted the changes in the mesenterial filaments and in the copepods. The results were the following.

Half an hour after the feeding in the digestive zone of the mesenterial filaments a number of red vacuoles are visible. Besides these vacuoles which have a fuchsin like hue (acid reaction), the remainder of the mesenterial filament, including the marginal part, has absorbed some neutral red which is unchanged in color. The copepod still has a deep red hue.

Gradually now the intensity of the color of the mesenterial filaments increases whilst the color (together with the muscles and other soft parts) is extracted from the copepod. At first the parts of the copepod which are lying against the mesenterial filaments lose their color, the distal parts of the appendages usually keep the neutral red longer than the central part of the animal. After two hours the mesenterial filaments are strongly colored, whilst the copepod is almost completely devoid of its soft parts and only the bare skeleton is left.

When the polyps are cut open to study the changes in the food and in the digestive organs the parts of the skeleton of the copepod usually disjoint. To avoid this the experiment can be modified in some respects. When a polyp, including the skeleton, is cut longitudinally into two halves, the digestive function of the mesenterial filaments may be studied under the microscope. The skeleton prevents the tissues from contracting strongly after the dissection and the mesenterial filaments remain clearly visible.

A copepod vitally stained with neutral red placed on the mesenterial filaments soon becomes partly enveloped by a few coils of these organs and the struggling movements of the animal soon come to an end. After some time (a quarter of an hour to half an hour) these coils withdraw from the food and then the digestive zone of their surface which has been in contact with the copepod has assumed a red color, thereby forming a striking contrast with the remaining parts of the mesenterial filaments. These colored parts, then apparently saturated with food, slowly bend away from the copepod and other coils of the same or a neighboring mesenterial filament take their place. The copepod remains intact as far as the external form is concerned till almost all of the colored internal tissues have been absorbed by the mesenterial

filaments. Afterwards usually the skeleton falls into pieces but not before almost the whole of the internal organs are dissolved. After about three hours the copepod is nearly completely devoid of its soft parts and the more or less disjointed skeletal elements are removed from between the mesenterial filaments.

This experiment, which can be easily followed under the microscope from the beginning till the end, gives a strong evidence for the view that besides intracellular digestion there is in *Astrangia* a secretion of an enzyme which dissolves the food in an extracellular way. Whilst the external surface of the copepod still is completely intact the greater part of the internal organs are already dissolved and ingested by the cells of the digestive zone of the mesenterial filaments. This disintegration of the soft parts of the copepod, as long as it does not yet fall into pieces, is only possible when a digestive fluid penetrates into it.

Probably in *Astrangia* a number of different digestive enzymes may be demonstrated as in the case of other cœlenterates (cf. Bodansky and Rose, 1922). The most effective of these enzymes is undoubtedly one which is comparable to the trypsin of other animals. This we may already expect in advance as the digestive vacuoles during the later period have an alkaline reaction. Moreover in all lower animals in which the proteolytic enzymes are studied they have a trypsin-like function (cf. Jordan, 1907*b*). I have made no elaborate experiments on the nature of the enzymes in *Astrangia*, but the few enzyme preparations tested showed that a trypsin-like enzyme is the chief factor for the disintegration of the food. Owing to the polyps being small the mesenterial filaments cannot easily be separated from the other parts of the polyps. The suspensions were made by pounding the tissues (chiefly consisting of the mesenterial filaments, extracted from a number of polyps) with sand to a mash. The latter was diluted with sea water and preserved with a few drops of chloroform. To equal parts of tissue suspension a piece of crab meat (previously boiled to destroy the blood enzymes of the crab it might contain) was added. Tests, containing crab meat in sea water with chloroform, were prepared in the same time. After four days the meat was partially dissolved. No positive results were obtained with the biuret reaction; the reaction with ninhydrin,



however, gave a definite purplish blue color, proving that the liquid contained amino-acids or peptids (cf. Howell, 1922). In the tests the crab meat was practically unaltered, the liquid gave only negative results with both reagents.

#### THE FOOD OF *ASTRANGIA* AND THE SYMBIOTIC ALGÆ.

Among my material there were a great number of colonies the polyps of which possessed numerous zoöxanthellæ in their entoderm. The feeding of these polyps therefore can to some extent be compared with that of reef-corals, which, as a rule, also contain large quantities of these unicellular algæ. On the other hand the polyps of *Astrangia* with the symbiotic organisms can be directly compared with those of the same species which are completely free of zoöxanthellæ.

In the literature there are comparatively few notes on the food of Madreporaria. According to the statements of many authors (cf. Duerden, 1902; Gardiner, 1902-03; Pratt, 1906; Walther, 1919) only very rarely remnants of food are found in the gastric cavity of madreporarian and also of alcyonarian polyps. Now the greater part of the researches on which these statements are based were made after the study of preserved material, and as I have already pointed out before (Boschma, 1924) this is at least partially due to the contraction of the polyps in the fixing fluid. When coral-polyps strongly contract the food-remnants which are in their gastric cavity usually are discharged through the mouth.<sup>4</sup> In living coral-polyps from the reefs in the East Indian region I usually found remnants of food in the gastric cavity embedded in mucus.

To ascertain the natural food of *Astrangia* I now proceeded in the same way and studied the specimens in the living state. As soon as the colonies were dredged I put them in sea-water on board the ship and left them undisturbed for about a quarter of an hour. Gradually the polyps now expanded more or less. After a mechanical stimulus (a slight touch with a forceps) they suddenly contracted, and at the same time the mouth opened widely. Usually then at the bottom of the gastric cavity a slight

<sup>4</sup> Some years ago Dantan (1921) also tried to explain this lack of food in the gastric cavity of anthozoans by the action of the preserving fluids which cause contraction and emptying of the gastric cavity.



amount of foreign material was to be seen, which could easily be extracted with a small forceps, without any damage to the polyps. The contents of the gastric cavity of 20 polyps consisted besides of undeterminable matter (detritus) of the following foreign organisms or parts of these: living diatoms (found in 12 polyps), diatom scales (found in 9 polyps), parts of higher algæ, usually in a partially decayed state (found in 4 polyps), foraminifera (found in 1 polyp), spicules of sponges (found in 5 polyps), parts of the stalks of hydroids (found in 2 polyps), a living nematode (found in 1 polyp), a dead larva of a polychæt worm (found in 1 polyp), parts of appendages or segments of the body of different smaller crustaceans (found in 10 polyps), shells of small bivalve mollusks (found in 3 polyps). Often also nematocysts or parts of these occur among the food-remnants and in the polyps which live in symbiosis with zoöxanthellæ invariably also these yellow algæ are to be found in the gastric cavity. Only very few polyps, when examined immediately after being collected, do not contain anything in their gastric cavity.

It is an interesting fact that in those polyps of *Astrangia* in which zoöxanthellæ occur in the entoderm cells, these algæ are always found in the remains of the food in the gastric cavity. These algæ are here in different stages of decomposition, owing to their being digested by the polyps. In this respect the polyps of *Astrangia* possessing zoöxanthellæ agree closely with reef-corals, in which the symbiotic algæ are also found in a partially digested state in the mesenterial filaments.<sup>5</sup>

There are, in general, two opinions concerning the food of reef-corals. One of these opinions was first put forward in a number of publications by Gardiner (1899, 1902, 1902-03, 1904b). This author found that the zoöxanthellæ form a large proportion of the food of all reef-corals, and maintains that many species of these corals even feed entirely on their symbiotic algæ. In a later publication Gardiner (1912) states that zoöxanthellæ are largely eaten by the coral-polyps when they require food, and further mentions that it is supposed that they catch and digest the small

<sup>5</sup> After my studies in Woods Hole I made some observations on the feeding of a few reef corals in the Bermuda Biological Station for Research. The polyps of these corals (*Isophyllia* and *Siderastrea*) in the natural state invariably contain partially decayed zoöxanthellæ in the digestive zone of the mesenterial filaments.

organisms of the superficial water of the sea. Hickson (1906) admits that the zoöxanthellæ may constitute a part of the food of reef-corals, but thinks it improbable that there are coral-polyps that feed exclusively on their yellow cells. In a recent work of Hickson the opinion is upheld that probably "the holozoic method of nutrition of the coral is supplemented by the holophytic action of the chlorophyll-bearing zoöxanthellæ" (Hickson, 1924, p. 21).

The other opinion on the food of reef-corals is expressed by Vaughan (1912, 1919) after a great number of feeding experiments on West Indian reef-corals. His conclusion is that the food of reef-corals solely consists of animal matter. This view is also supported by Mayer (1918), who based his opinion on Vaughan's experiments and on the statement of Duerden (1904) that the polyps of *Siderastrea* are easily kept alive with meat of crabs and other animals.<sup>6</sup>

Vaughan's opinion was first criticized by Gravier (1913). According to Gravier it is improbable that coral-polyps live exclusively on animal matter, for their symbiotic algæ are undoubtedly also a factor in their nutrition as direct food or indirectly by the supply of carbohydrates. After the study of the contents of the cœlenteron of many coral-polyps I also (Boschma, 1924) came to a conclusion differing from Vaughan's. I found that the food-remnants in the gastric cavity of the polyps of reef-corals always contained zoöxanthellæ in various stages of disintegration, undoubtedly owing to their being digested by the polyps. Besides these algæ also animal matter was found in the remains of the food, rarely in the smaller polyps, more often in the larger polyps.

It is generally understood that the products of the photosynthesis of the zoöxanthellæ assist in the nutrition of the cœlenterates containing these algæ (Buchner 1921, 1924; Hickson, 1924). In many anthozoans which harbor large quantities of these unicellular algæ even a degeneration of the food-capturing

<sup>6</sup> Besides the facts on the food of reef corals there are some statements on the food of madreporarian corals which do not contain zoöxanthellæ in their entoderm. Some notes on the food of deep-sea corals are found in the publications of Gravier (1920) and Boschma (1924). In the latter article also data on the food of species of *Dendrophyllia* from shallow water are given. Cf. also Gardiner's articles on *Cænopsammia* and *Flabellum*, cited above.

and digestive portions of the polyps has taken place, as in *Sclerophyllum gardineri* (Pratt, 1903, 1906) and in *Galaxea musicalis* (Matthai, 1914).

The feeding of cœlenterates on their symbiotic algæ as a whole has been reported in a few cases only. In young medusæ of *Aurelia aurita* Friedemann (1902) has mentioned instances of this phenomenon. According to Pratt (1903) in the polyps of the alcyonarian coral *Sclerophyllum* frequently zoöxanthellæ are observed in a partially digested condition in the mesenterial filaments (cf. also Pratt, 1906). Digestion of zoöxanthellæ in the polyps of reef corals is recorded by myself (1924). Probably the irregular green corpuscles which McMurrich (1889) found among the zoöxanthellæ in the digestive part of the mesenterial filaments of zoanthids were also such partially digested algæ. In *Velella* the zoöxanthellæ of the developing larvæ which migrate to deeper water probably serve as a source of food-substance only, as their photosynthetic action is impeded by the darkness (Woltereck, 1904).

According to Fulton (1921, 1922) the association of cœlenterates with their zoöxanthellæ probably is of a parasitic nature (the polyps being the parasites of the algæ), as during starvation sea-anemones feed upon the unicellular algæ rather than upon their photosynthetic products.

Besides their feeding on the zoöxanthellæ or on their products of assimilation the polyps also derive some profit from the algæ living in their tissues, as they are a source of oxygen for the polyps. The investigations of Brandt (1883), Trendelenburg (1908) and Pütter (1911) prove that actinians with zoöxanthellæ may derive a large part of their oxygen from these algæ, and that actinians with zoöxanthellæ can better resist unfavorable circumstances than those which do not harbor unicellular algæ. Probably the algæ also use nitrogenous waste products of the polyps, which may be one of the profits the zoöxanthellæ derive from the association with the cœlenterates.

In the case of *Astrangia* zoöxanthellæ are always found in the digestive region of the mesenterial filaments of the polyps which are infected with these algæ. In contradistinction to the algæ in the entoderm of the oral disk and the tentacles those in the

digestive zone have lost their yellow color to some extent. White spots then appear in their interior, whilst the contents are contracted and often have assumed a brownish hue. All different stages of the decomposition of these algæ are found in the digestive region, indicating the probability that they are being digested here. In the polyps of *Astrangia* which are not infected with zoöxanthellæ these algæ never occur in the digestive region of the mesenterial filaments. Consequently the zoöxanthellæ do not form a necessary part in the feeding of the polyps. A priori it is highly probable that they are digested on account of the lack of other food. We may therefore expect that, when food of other origin in great abundance is given to the polyps, the cells of the digestive zone of the mesenterial filaments will ingest no more zoöxanthellæ.

In fact these changes of the contents of the digestive region are easily accomplished. For my experiments I used dark brown polyps, the digestive organs of which before the artificial feeding contained a large quantity of partially decayed zoöxanthellæ. These polyps now were repeatedly fed with crab meat and already after three days the digestive region of the mesenterial filaments was almost completely devoid of zoöxanthellæ. Still a few yellow-brown corpuscles were recognizable as strongly decayed algæ, but it was evident that after the feeding of the polyps with crab meat no more algæ were ingested. The zoöxanthellæ which were already present in the digestive organs were completely digested. In this way the polyps can be compelled to change the nature of their food. It is obvious from these facts that the zoöxanthellæ are ingested by comparatively hungry polyps only. In the natural state the amount of food available for the polyps seems to be rather scanty and therefore they usually derive a part of their food from the digested unicellular algæ.

Van Triët (1919) has described a similar phenomenon in fresh-water sponges (*Spongilla*), which contain zoöchlorellæ. These unicellular algæ, which are continually imported from the surrounding water, constitute a very important source of food for the sponges. When circumstances are favorable the algæ are killed and digested by the sponge only in part, the rest of the imported algæ then can live on, photosynthesize and multiply in the tissues.

On the other hand in less favorable circumstances the whole of the imported algæ together with those already present in the tissues of the sponges are digested.

#### INFECTION OF COLORLESS POLYPS WITH ZOÖXANTHELLÆ.

In my experiments I kept the colonies of white polyps separated from those with brown polyps (brown on account of their zoöxanthellæ). As the white polyps did not become infected during a month's time there is little evidence that infection occurs through free zoöxanthellæ in the sea water. In a number of polyps I produced an artificial infection with zoöxanthellæ by feeding them with portions of the soft parts of dark brown polyps. Especially those parts of the brown polyps which contained a multitude of these algæ (the tentacles and the oral disk) were used for this experiment. The tissues were torn to minute pieces and thoroughly mixed with pounded crab meat. It was necessary to add meat to the tissue preparations for otherwise the polyps refused to feed upon it.

Soon after feeding a number of zoöxanthellæ had detached from the crab meat and were floating free in the gastric cavity of the polyps and sometimes even the tentacles assumed a light brown color by the algæ which had penetrated into their cavity. A large part of the zoöxanthellæ from the feeding mixture were ingested together with the meat by the mesenterial filaments. After three days the digestive region of these organs was crowded with algæ. Some of these were already partially digested, for white spots had appeared in the originally evenly yellow colored interior.

Some of the cells which a very short time after the feeding are found in the cavity of the tentacles penetrate into the entoderm cells of these organs. Here they quickly multiply by division into two halves. The following numbers clearly illustrate the quick increase in number of the zoöxanthellæ in the tentacles of recently infected polyps. In one polyp on the day after the infection a cut-off top of a tentacle contained 6 symbiotic algæ, three days after the infection a top of a tentacle of the same length contained 23. For another polyp the number of zoöxanthellæ in the entoderm of different distal parts of tentacles of about equal

size was: 1 day after the feeding 4 and 5; 2 days after the feeding 9, 16, 2, 21 and 12. In a third polyp the number of the algæ in tentacular extremities of approximately equal size was: 3 days after the feeding 30, 28, 22, 17 and 27; 9 days after the feeding 72 and 53. Consequently the zoöxanthellæ in the entoderm cells after the infection gradually had increased in number, though the change in color of the polyp could not yet be observed at first sight, for in moderately brown polyps extremities of tentacles of the same size as those cut-off in my experiments contain far more than 1,000 zoöxanthellæ.

After the feeding with the mixture of crab meat and zoöxanthellæ the infected polyps were fed several times with crab meat to keep them in a well-fed state. In hungry polyps the zoöxanthellæ perhaps might divide less rapidly.

I could not continue these experiments of infection for more than 9 days, but I am convinced that in this way a white polyp may be changed into a brown one crowded with zoöxanthellæ in the entoderm cells. Probably in nature the infection may take place (though rarely) in a similar way. When the polyps capture an animal which contains zoöxanthellæ some of these may find their way to the entoderm of the tentacles or the oral disk. After division these cells may infect the neighboring cells and at last the whole of the entoderm of the colony. This manner of infection in all probability only rarely occurs. As in other coral polyps with symbiotic algæ the greater part of the infected polyps of *Astrangia* undoubtedly have originated from planulæ which already obtained the symbiotic algæ before hatching. This view is supported by the fact that all the infected polyps of the Woods Hole region were found in a comparatively restricted locality.

#### SUMMARY.

The feeding reactions of *Astrangia* in general consist of four actions: muscular action of the tentacles, muscular action of the central part of the oral disk, secretion of mucus by the oral disk, and ciliary action of the stomodæum.

The reaction of the food-vacuoles in the digestive region of the mesenterial filaments immediately after the feeding is acid; after about two days the reaction changes to alkaline. In this alkaline



period in all probability the digestion takes place. There is sufficient evidence that besides intracellular digestion a secretion of a digestive fluid (a trypsin-like enzyme) occurs.

In the polyps of *Astrangia* which contain zoöxanthellæ in their entoderm these unicellular algæ furnish a part of the normal food of the polyps: a quantity of these algæ are digested in the mesenterial filaments.

Polyps of *Astrangia* without zoöxanthellæ can be easily infected with these algæ by feeding them with crab meat mixed with parts of the tissues of strongly infected polyps.

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